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CARIBBEAN ISLAND ZOOGEOGRAPHY: A NEW APPROACH USING MITOCHONDRIAL DNA TO STUDY NEOTROPICAL BATS

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Abstract

Genetic analysis of animal mitochondrial DNA is a new and valuable addition to the battery of techniques available to zoogeographers. This paper describes characteristics of mitochondrial DNA (mtDNA) that make it applicable for the study of island zoogeography.

Some traditional zoogeographic questions are examined using mtDNA from the Neotropical fruit bat, *Artibeus jamaicensis*. The specific questions are: 1) To what extent are island populations isolated (that is, does interbreeding occur between the insular subspecies)? 2) Can a single founding female account for the mitochondrial genomes on specific islands in the Antilles? 3) Is there a correlation between the genomic diversity of an island population and the size of the island or the distance from the mainland?

The mitochondrial genome in *Artibeus jamaicensis* is approximately 16,000-16,500 base pairs. Three major mtDNA groups (designated J, SV, and G), separated by 8 to 17.2 percent divergence in nucleotide sequence, were identified in Antillean *Artibeus jamaicensis*. The J and SV groups each includes two maternal lineages and the G group is represented by three lineages. The sequence divergence between the mtDNA groups is unusually high for conspecific mammals. Either the mtDNA in *Artibeus jamaicensis* can be traced to three relatively old origins or alternatively chiropteran mtDNA evolves at a faster rate than mtDNA in rodents and primates.

Populations on five of eight islands were clearly derived from multiple maternal ancestors. The greatest genetic diversity (judged by numbers of lineages and sequence divergence) was found in *A. j. trinitatus* on Grenada, which probably is a reflection of the proximity of the island to the South American mainland. Genetic diversity did not correlate with island size. One particular mtDNA lineage, J-1, was the most widespread, being found from Jamaica to Barbados and Grenada. These bats trace their ancestry to a common female, underscoring the dispersal and colonization capabilities of *A. jamaicensis*. MtDNA data and paleontological evidence suggest these bats reached the Antilles recently, probably in conjunction with replacement of the xeric Pleistocene environment. The island distribution of mtDNA genotypes somewhat corresponds to the currently recognized subspecies: 77 percent of *A. j. jamaicensis* carry the J mtDNA genotypes;

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93 percent of *A. j. schwartzi* carry the SV mtDNA genotype; and 60 percent of the *A. j. trinitatus* carry the G mtDNA genotypes.

The geographic distribution of specific mtDNA genotypes on different islands documents gene flow between and among islands. For example, the data show that female bats with a common maternal ancestor have moved among the islands of St. Lucia, St. Vincent, Barbados, and Grenada even though they would be required to fly up to 150 km across open ocean. MtDNA and distributional data are used to postulate that the Grenadine islands, between Grenada and St. Vincent have been a partial barrier to gene flow, possibly because of their small size and xeric habitats.

Introduction

The occurrence, zoogeography, and speciation of mammals in the Caribbean have been studied for decades. Overviews of colonization and extinctions have been recently presented by Morgan and Woods (1986), Pregill (1981), Pregill and Olson (1981), and other authors in this volume. Most of what we know about Caribbean mammals has come from conventional data sources such as fossils and museum specimens. With previously available data, it has been difficult to test certain long standing hypotheses such as the following: Do most populations arise from single rather than multiple genetic founding events? Do some island populations receive periodic infusions of new DNA? Can species survival on islands be predicted by island size, ecological diversity, geographic location, or is it the result of chance?

Recent developments in molecular biology make it possible to consider zoogeographic problems from a new perspective--high resolution genetic analysis of individuals. In particular we think that restriction endonuclease analysis of mitochondrial DNA (mtDNA) will improve our understanding of zoogeography of the Caribbean. Our goals with the present paper are to: 1) provide background information about mtDNA analysis; 2) share some practical information about how we are applying the technique to a particular project in the Caribbean; and 3) discuss some recently acquired data in a zoogeographic context.

Mitochondrial DNA has specific characteristics that make it appropriate for certain zoogeographic problems where other methods have been either not particularly helpful or insufficient. Mitochondria are found in virtually all cells throughout an organism. Because each mitochondrion contains at least one copy of the small, circular mitochondrial genome (approximately 15,700-19,500 base pairs in animals; Brown 1983, 1985), and because there are usually many mitochondria per cell (approximately 1,000 in an average liver cell), isolation of the mitochondrial fraction from a tissue supplies the investigator with many identical copies of a small genome. The mitochondrial genome is inherited from the mother (Dawid and Blackler 1972; Hecht et al. 1984), allowing one to determine maternal ancestry and, potentially, to trace the living members of a species to a single female founder (Cann et al. 1987).

The mitochondrial genome evolves at a faster rate than the nuclear genome (Brown et al. 1979; Vawter and Brown 1986). This characteristic, together with its mode of inheritance, make mtDNA valuable for certain kinds of studies, particularly of congeners or conspecifics. The technique has been successfully applied to several kinds of systematic and evolutionary problems, including: relationships among higher primates (Hixson and

Brown 1986); history of the human species (Brown 1980; Wallace et al. 1985; Cann et al. 1987); phylogeny of the genus *Equus* (George and Ryder 1986); pocket gophers, *Geomys* (Avise et al. 1979a; Laerm et al. 1982); ground squirrels, *Spermophilus* (MacNeil and Strobeck 1987); deer mice, *Peromyscus* (Avise et al. 1979b; Lansman et al. 1983; Nelson et al. 1987; Ashley and Wills 1987); several species of the mouse, *Mus* (Boursot et al. 1985; Gyllensten and Wilson 1987); parthenogenetic lizards (Wright et al. 1983); anurans (Spolsky and Uzzell 1986; Lamb and Avise 1986; Carr et al. 1987); crickets (Harrison et al. 1985); and zoogeography of fish (Bermingham and Avise 1986; Avise et al. 1986).

In order to perform the analysis, it is necessary to isolate the mtDNA and cleave it with restriction endonucleases. These enzymes cut all identical mitochondrial genomes into a set of fragments, which can be separated by size using gel electrophoresis (Nathans and Smith 1975). Restriction enzymes can be selected that recognize different palindromic sequences and cut the chromosome in different places. Therefore, each enzyme creates a different pattern of fragments. By comparing fragments obtained with different enzymes, singly and in combination, the relative location of the recognition sites for a particular enzyme can be determined and the genome can be mapped. All individuals from the same maternal lineage will have identical restriction enzyme recognition sites within the chromosome. The longer the time since two individuals shared the same maternal ancestor, the more divergent their restriction fragment patterns are likely to be. The amount of divergence between two different genotypes can be estimated mathematically by comparing the number of shared versus nonshared restriction enzyme recognition sites.

The above approach can be directly applied to zoogeographic problems. For example, in theory one could locate possible geographic sources of island populations by tracing maternal lineages back to a mainland locality. Because the rate at which mitochondrial DNA evolves can sometimes be estimated, it might be possible to determine the minimum time since the invasion and colonization of an island. Several examples of the use of mtDNA to investigate historical zoogeography have been published recently (Boursot et al. 1985; Ashley and Wills 1987; MacNeil and Strobeck 1987) and discussed in detail by Avise et al. (1987).

What group of mammals might serve as a good model for testing the use of mtDNA analysis in studies of island zoogeography? First, the group should be reasonably widespread so that an adequate sample of islands can be compared (see Hastings 1987). Second, there should be some indication that breeding is non-uniform; that is, a geographic pattern of phenotypic differences is desirable. Third, conventional taxonomic data should be available. Fourth, the animals within the study group should be closely related. High-resolution mtDNA analysis is more likely to produce meaningful results with conspecifics or congenerics.

On the basis of these criteria we selected a species of the Neotropical phyllostomid fruit bat, *Artibeus* for our study. Many zoogeographers disregard bats when discussing island populations of mammals (De Beaufort 1951; Darlington 1963; Heaney 1985, 1986) because the flying capabilities of bats appear to lend them easy access to islands. However, systematists have often shown that the distribution of bats within archipelagos is nonuniform. Megachiropteran bats in the Solomon Islands demonstrate striking patterns of morphological variation between adjacent islands and the archipelago is inhabited by endemic genera and species (Phillips 1966, 1968). In Caribbean populations of microchiropteran bats there also are endemics as well as many examples of complex distributional patterns and interisland variation in size, color, and dentition (Koopman 1958, 1968, 1976; Jones and Phillips 1970, 1976; Jones, 1978; Baker and Genoways 1978; Swanepoel and Genoways 1978; Genoways et al. 1981; Ottenwalder and Genoways 1982). These data, obtained by the most commonly used techniques, appear to support the argument that bats can, and do, become reproductively isolated on islands.

Fruit bats of the genus *Artibeus* occur throughout most of Latin America and the Caribbean. The conventional view is that the Antilles are populated by two species (Koopman 1976; Jones 1978). One, *A. jamaicensis*, is very common and geographically widespread. It also varies both morphometrically and morphologically in the Caribbean (Koopman 1968; Jones and Phillips 1970). The other species, *A. lituratus*, is thought to occur only on two islands in the Lesser Antilles. Many specimens have been collected on Grenada and one specimen is reported from St. Vincent (Jones 1978, present volume). Morphologically, behaviorally, and ecologically these two species are very similar; they probably use essentially the same nutrient resources and have similar reproductive cycles (Gardner 1977; Wilson 1979). Their distribution in the Caribbean appears to qualify as an example of competitive exclusion under the criteria outlined by Hastings (1987).

Methodology

Specimen collection

The bats used in this study were collected by mist netting at night in flyways, over water sources, and at cave entrances. Some were sacrificed (with T-61 euthanasia solution) the next morning and their tissues removed and processed. Others were brought alive to our laboratory (with appropriate permits) and processed there. Voucher specimens were deposited in the collections of the Carnegie Museum of Natural History, Pittsburgh, PA, the University State Museum, University of Nebraska-Lincoln, NB, and The Museum, Texas Tech University, Lubbock, TX.

The most appropriate tissue for mtDNA analysis varies somewhat from species to species. Liver is often used, for example, from sheep and goats (Upholt and Dawid 1977) and from mice and gophers (Avise et al. 1979a, 1979b). However, the liver presents problems in some mammals (for example bats, our data and G. McCracken, pers. comm.; and voles, our data and Tegelstr?m [1986]). Generally, animals are homoplasmic with respect to the mtDNA from different tissues (Avise and Lansman 1983), however, heteroplasmy of mtDNA has been described (Solignac et al. 1983; Hauswirth et al. 1984; Densmore et al. 1985; Bermingham et al. 1986). Tissues used successfully include heart, liver, kidneys, and ovaries from lizards (Wright et al. 1983), liver and/or heart from fish (Avise et al. 1986; Bermingham and Avise 1986), placenta from humans (Brown 1980; Cann et al. 1987), liver or brain from dairy cows (Hauswirth et al. 1984), and in the case of small organisms the entire individual (Solignac et al. 1983; Harrison et al. 1985).

We used liver, kidney, heart, and skeletal muscle for our study. Kidney is the most satisfactory, but it must be supplemented with other tissues to provide sufficient DNA for mapping. Laboratory preparation of fresh tissue gives the best yield of mtDNA. Tissue from field-sacrificed specimens was either packed in cryotubes and immediately frozen in liquid nitrogen, or homogenized in eight volumes of buffer (0.25 M sucrose, 10 mM Tris-HCl[pH 7.5], 1 mM ethylenediamine-tetraacetate, 8.3 mM NaCl), centrifuged to remove the nuclei and cellular debris, and incubated for 30 min with Proteinase K (50 μg/ ml supernatant) before freezing in dry ice. **Note added in proof:** Southern blot hybridization and the polymerase chain reaction (e.g. Kocher et al. 1989) are now used to study mtDNA. These techniques allow collection of information from partially degraded specimens.

Laboratory methodology

The details of the techniques we used are presented elsewhere (Brown 1980; Wright et al. 1983) and will not be repeated here. Instead, we will outline the techniques and focus on the analysis.

Mitochondria are separated from nuclei and other cellular debris by differential centrifugation and then purified through sucrose. The mitochondria are lysed by addition of sodium dodecyl sulfate (SDS). Many proteins are removed by precipitating the sample with saturated CsCl at 4½C. The intact, circular, mtDNA is separated from contaminating nuclear DNA and nicked or otherwise damaged mtDNA by cesium chloride density gradient centrifugation. The mitochondrial band is removed, dialyzed to remove the salts and dye necessary for the centrifugation, and precipitated with ethanol. The purified DNA is then ready for the analysis.

Small amounts of DNA are mixed with a restriction endonuclease in the appropriate buffer. It is the availability of a variety of these restriction enzymes that is the key to mtDNA analysis. These enzymes recognize specific base pair sequences and cleave the DNA within the recognition sequence (Nathans and Smith 1975). For example, the enzyme *Bam*H I recognizes the six base sequence 5'..GGATCC..3'. The recognition sequences are palindromes, which means that the sequence is the same on the complementary strands of DNA. The enzyme *Hin*d III recognizes 5'..AAGCTT..3', *Mbo* I recognizes 5'..GATC..3' and so on. Because these enzymes only recognize a specific sequence, they will cleave identical pieces of DNA, in our case mitochondria from a single individual, into a specific set of fragments that can be separated and sized by agarose or acrylamide gel electrophoresis (Southern 1979) or electron microscopy (Brown and Vinograd 1974).

We used the following restriction enzymes for the present study: *Bam*H I, *Bgl* II, *Pvu* II, *Hin*d III, *Eco*R I, *Pst* I, *Sal* I, *Xba* I, and *Xho* I. The mitochondrial DNA lineages are defined by the set of restriction fragments obtained after digestion, electrophoretic separation, and autoradiography. Individuals whose mtDNA has the same pattern of restriction endonuclease recognition sites are within the same maternal lineage. An alteration of the restriction enzyme cleavage pattern indicates that one or more mutations occurred in the female ancestors. The more differences between or among the genotypes, the longer the time since the individuals shared a common maternal ancestor.

It is tempting to think that the restriction fragment pattern obtained following gel electrophoresis and autoradiography is sufficient to calculate the amount of divergence. However, not all fragments that migrate the same distance on a gel represent homologous fragments. If one attempts to determine sequence divergence using just the restriction fragment sizes, the result will only be a minimum value. To be certain that two similar size fragments from different genomes are from homologous regions one must map the fragments (Nathans and Smith 1975). This is usually achieved by digesting the same mtDNA with two or more enzymes before electrophoresis. These data are then used to create a map of the genome (Fig. 1). Mapping can be done with the aid of several available computer programs (Fitch et al. 1983; for review, Bishop 1984). In *Artibeus* we found that some enzymes produced several similar sized, seemingly homologous, fragments in different genomes. After mapping, however, it was obvious that the fragments were not homologous, just coincidentally similar in size (Pumo et al. 1988).

Several formulae have been developed to estimate nucleotide sequence divergence based on restriction fragment information. The method of Upholt (1977), which uses unmapped fragment data, gives only a minimum divergence and could be potentially misleading because similar sized fragments are not necessarily homologous. Equation 16 (Nei and Li 1979) is currently widely used to calculate mtDNA sequence divergence from mapped restriction sites. The two disadvantages with this procedure are that no variance can be calculated and one must either know (at present unlikely) or estimate an alpha value. The values obtained with mapped restriction site data and equations 9 and 11 (Nei and Tajima 1983) are more conservative than equation 16 and allow estimation of the variance. The values obtained with equation 9 are the values referred to in the text. Such data can be used for pairwise comparisons of lineages and phylogenetic interpretations. The divergence can also be used to estimate the time of lineage separations (Brown et al. 1979; Cann et al. 1987).

The mitochondrial genome in *Artibeus jamaicensis*

The mitochondrial genome in *Artibeus jamaicensis* is approximately 16,000-16,500 base pairs (bp) in length. The mtDNA genome in *A. jamaicensis* was analyzed with restriction endonucleases that recognize specific 6-bp and 4-bp sequences (Pumo et al. 1988). For the analysis in this paper, we concentrated on 6-bp cutters. The enzymes *Bam*H I, *Bgl* II, and *Pvu* II each recognize one to four restriction sites, *Xba* I recognizes about five, and *Hin*d III recognizes six to ten sites in the *A. jamaicensis* mitochondrial genomes presented here. Three other restriction enzymes, *Eco*R I, *Pst* I, *Xho* I, and *Sal* I each recognize from 0 to 2 sites in the individuals surveyed thus far. The most interesting and complex data have been obtained with Hind III. Each of the major *A. jamaicensis* mtDNA groups known from the Antilles can be recognized with this enzyme because each has its own characteristic restriction fragment pattern that serves as a signature for the genotype (Fig. 1).

The restriction maps for five of the mtDNA lineages are shown in Fig. 1. The maps are linearized at a conserved *Bam*H I restriction site. When this is done numerous similarities and differences among the genotypes are immediately apparent. With this data set the five lineages clearly fall into three distinct groups of mtDNA genotypes based on the number of apparently conserved restriction sites (Fig. 1). Within each group there are less than three site differences, whereas between groups there are at least nine restriction site differences. The differences and similarities among the genotypes appear to be positioned throughout the genome, with no one large region more variable than another. Figure 1 contains representative lineages from each of the three major groupings of mtDNA genotypes, designated J, SV, and G recovered from 164 specimens of *Artibeus jamaicensis* from the islands of Jamaica, Puerto Rico, Anguilla, St. Vincent, St. Lucia, Barbados, Bequia, and Grenada. The numbers of specimens in each mtDNA group and the islands from which they were collected are summarized in Table 1.

All mtDNA groups found in specimens of *Artibeus jamaicensis* are represented in the Antilles by two or more maternal lineages. The J mtDNA group can be divided into a J-1 (84 individuals) and a J-2 (two individuals) lineage; J-2 has only been identified on the island of Jamaica, whereas the J-1 lineage is widespread in the Antilles. The J-2 lineage lacks a *Hin*d III site (Fig. 1) present in the J-1 lineage (Pumo et al. 1988). The SV group also contains two lineages. SV-1 predominates and was isolated from individuals on Barbados, St. Lucia, St. Vincent, Bequia, and Grenada. SV-2 was isolated from only one individual living on Grenada. The restriction site map for SV-2 is identical to SV-1 for all enzymes tested except *Hin*d III. SV-2 lacks two sites found in SV-1, and SV-2 has one site which is not present in SV-1. The G mtDNA group is represented by at least three lineages in the Lesser Antilles. On Grenada, G-1 is represented by six individuals and G-3 by three individuals. G-2 is represented by nine individuals, one from St. Vincent and the remainder from Grenada. The G-2 lineage differs from G-1 in having a *Sal* I restriction site (Fig. 1). The G-3 lineage lacks one *Hin*d III site present in G-1 and G-2 and does not have a *Sal* I site.

Restriction enzyme maps representing individual lineages can be used to estimate the overall sequence divergence between each lineage. An assessment of the difference between genotypes can be gained by using mapped restriction endonuclease cleavage sites (Table 2). Nei and Li's (1979) formula 16 with an alpha = 2 is currently widely used for obtaining estimates of sequence divergence (Carr et al. 1986). Our discussions are based on comparisons calculated with Nei and Tajima's (1983) more conservative formulae 9 and 11. On this basis the J-1 and J-2 lineages are estimated to have diverged only 0.4 percent, whereas the J-1 and SV genotypes have 8 percent sequence divergence. The greatest amount of sequence divergence is between the SV and G-2 genotypes, which were estimated to differ by 13.9 percent. Although the 1979 formulae generate larger numbers, the qualitative results are identical with both procedures.

What is the significance of the extensive differences between each of the three major mtDNA groups identified in Antillean *Artibeus jamaicensis*? The best comparative data come from other mammals for which restriction maps are available and where the data have been analyzed by means of Nei and Li's (1979) method. On this basis breeds of horses have been estimated to differ by about 0.55 percent, whereas species of *Equus* differ by up to 7.5 percent (George and Ryder 1986), and California black-tailed deer (*Odocoileus hemionus*) and South Carolina white-tailed deer (*O. virginianus*) differ by 6.9 percent (Carr et al. 1986). Humans have been investigated in considerable detail and the mtDNA differs among lineages by about 0.32 percent (Brown 1980; Cann et al. 1987). Thus, the estimated differences between the J-1 and J-2 lineages and among the G mtDNA lineages are similar to intraspecific mammalian "breed" divergence, whereas the estimated differences among the three mtDNA genotype groups (8.2-13.9 %) exceeds that for most reported intraspecific comparisons among mammals. However, Honeycutt et al. (1987) report intraspecies variation in African mole rats, *Cryptomys hottentotus*, as high as 20 percent. Estimates of sequence divergence in the range of 10-20 percent are similar to those reported for many interspecific comparisons among frogs of the genus *Xenopus* (Carr et al. 1987). The high values for frogs are thought to be the result of divergence accumulated over a great period of time. The previously reported values for mammals are thought to be in keeping with Brown's (1980) calculation that mtDNA sequences in primates evolve at approximately 1 percent per million years.

Does the mitochondrial genome in *Artibeus* evolve at an extremely fast rate or is there some other explanation for the level of sequence divergence estimated from our data? One explanation is that *Artibeus* is a very old genus of phyllostomid bat. Smith (1976) concluded that most of the major microchiropteran families were well-established by at least the middle Oligocene or Miocene and Straney et al. (1979) used allozyme data to estimate that the Phyllostomidae diversified about 40 myBP. The possibility exists, therefore, that the mtDNA genotypes isolated from Antillean *A. jamaicensis* can be traced back to an ancient common ancestry. Another possibility is that the estimates of divergence for these genotypes are greater than expected due to insertions, deletions, or rearrangements in parts of the mitochondrial genome. In making our calculations, restriction sites that differed by more than 400 bp were regarded as non-homologous. Additions or deletions of fairly large (500-700 bp) sections in particular regions of the three genotypes would bring some restriction sites into apparent alignment. Length variation due to additions and deletions in the mtDNA of conspecific animals has been reported in non-mammalian vertebrates (Brown 1985) but its occurrence in the mtDNA of *Artibeus* has not yet been verified.

In summary, three different explanations can be offered for the large estimates of sequence divergence: 1) the mtDNA in *Artibeus* evolves at a significantly faster rate than in other studied mammals; 2) the extant mtDNA lineages are relatively ancient; and 3) evolution of the mitochondrial genome in *Artibeus jamaicensis* has involved additions, deletions, or rearrangements that affect the alignment of restriction maps and exaggerate the degree of sequence divergence. Until more data are available it will be difficult to favor any one of these three hypotheses about the evolution of the mitochondrial genome in *Artibeus*.

Zoogeography

Restriction endonuclease analysis of mtDNA is proving to be valuable to zoogeographic investigations. Some previous applications of the technology have advanced our understanding of human history (Wallace et al. 1985; Cann et al. 1987), have documented the dynamic interaction between two species of deer that occurred when their ranges came into sympatry due to alteration of environment (Carr et al. 1986), have examined the pattern of colonization of the northern Rocky Mountains by Columbian ground squirrels (MacNeil and Strobeck 1987), and colonization of Scandinavia by house mice, *Mus* (Gyllensten and Wilson 1987). The geographic distribution of mtDNA lineages can be interpreted in terms of other, independent, events to gain an insight into historical zoogeography. For example, Gyllensten and Wilson (1987) related the movement of *Mus* into Scandinavia with human agricultural activities and Boursot et al. (1985) correlated North African distribution of mtDNA lineages of *Mus spretus* with the northward expansion of desert and differentiation of isolated floral zones.

Analysis of mtDNA might be useful for island studies because the genome evolves rapidly and it might be possible to trace the dispersal history of individual maternal lineages. Ashley and Wills (1987) applied mtDNA analysis to deer mice (*Peromyscus maniculatus*) on the Channel Islands. Plant and colleagues (1989) used mtDNA analysis to study variation in meadow voles (*Microtus pennsylvanicus*). Both groups compared island populations to the mainland, estimated time of colonization, and compared intrapopulation genetic variation. Ultimately this same type of analysis might also be used to test hypotheses about mainland origins of Caribbean populations of mammals, depending on the availability of specimens and the presence of related maternal lineages on mainland areas.

Colonization and intraisland genetic diversity

Each of the island populations of *Artibeus jamaicensis* consists of descendants of some founding individuals. One could argue that island colonization usually has resulted from small numbers of individuals who reproduced successfully and established a long-term population. The probability of successful colonization from a small propagule is very high if the island can support a large population ($K > 1,000$) and the species has a per capita birth rate ( ) greater than the death rate (μ) (MacArthur and Wilson 1967). Once an island population reaches, or at least approaches, K, it then would seemingly be difficult for new arrivals to find living space and food. Insofar as fruit bats are concerned, it seems reasonable, *a priori*, to think that each Antillean island offers finite opportunities for success; the islands are limited in size, diversity, total fruit production, suitable roosting sites, and availability of fresh water.

With few exceptions, allozyme analyses have confirmed that island populations of small mammals have less genetic variability than mainland populations (Kilpatrick 1981; Berry 1986). Interestingly, one of the possible exceptions is the population of leaf-nosed bats, *Macrotus waterhousii*, on Jamaica (Greenbaum and Baker 1976). However, this exception might have been exagerated by data from a single locus (Greenbaum and Baker 1976). The more usual finding with allozyme comparisons, reduction in genetic variability, is thought to be the result of a founding effect, which involves intense inbreeding leading to increased frequency of monomorphic alleles and common mainland alleles and the loss of rare, or low frequency, mainland alleles (Kilpatrick 1981). Some authors (e.g., Mayr 1963) have argued that genetic diversity might be gradually restored and others (e.g., Slatkin 1987) have pointed out that occasional genetic input from new arrivals could have a significant "creative" evolutionary effect.

Because it is maternally inherited and not subject to recombination, the mitochondrial genome is not affected by the same factors as the nuclear genome. An island population founded by females carrying highly divergent mtDNA could exhibit a significant decrease in variability in the nuclear genome without any effect on the mitochondrial genotypes. Additionally, an island population might, over time, evolve a number of new mtDNA genotypes, each being derived from the founding mtDNA genotype(s). A long term, isolated, island population thus might carry some number of endemic mtDNA genotypes. However, the survival of new or old mtDNA genotypes is dependent on reproductive success within female lineages and is far from being assured. Indeed, lineage survival might be unlikely over a lengthy period of time (Avise et al. 1984). Consequently, the absence of multiple mtDNA genotypes in an island population could be the result of lineage extinction or, alternatively, a seemingly endemic mtDNA genotype could be the result of its extinction elsewhere. Some mtDNA data thus are difficult to interpret. On the other hand, if two or more geographically wide-spread mtDNA genotypes are also found in an island population, it would be reasonable to think that females in each mtDNA lineage either were part of the founding group, or immigrated separately into the island population, or both.

Another consideration is the amount of sequence divergence in mtDNA within an island population. Large amounts of divergence (e.g., >4%) would require a considerable period of time if mtDNA evolves at the rate of one percent per million years (Brown 1980; Higuchi et al. 1987). Because of stochastic lineage extinction, there is little probability of this much divergence accumulating in a small island population (Avise et al. 1984). For example, in a model population founded by females in two lineages, producing female offspring according to a Poisson distribution with a mean of 0.9, computer-generated data predict mtDNA lineage extinction in about 25 generations (Avise et al. 1984). The data generated by Avise et al. (1984) also predict that once a population reaches carrying capacity (K) of n size (K=n), within 4n generations all of the descendants will trace their ancestry to a founding female. Consequently, unless new females carrying different mtDNA genotypes had immigrated independently into an island, one would expect to detect either a single lineage or, at best, several very closely related lineages (i.e., <1 % sequence divergence) within a long-term insular population (Avise et al. 1987).

The founding of an island population can be viewed in terms of the arrival of a group of individuals, or a single individual, from whom future generations are derived. In terms of mtDNA, the arrival of females carrying divergent mtDNA genotypes might be viewed as separate genetic founding events, at least insofar as mtDNA lineages are concerned. Based on this and the foregoing discussion, we would hypothesize that some Antillean island populations of *Artibeus jamaicensis* resulted from several genetic founding events, probably in the form of periodic or occasional immigrations. For example, on Barbados and St. Vincent we found representatives of two female lineages, SV-1 and J-1. Their mtDNA exhibited so much sequence divergence (8%) that we placed their genotypes in separate mtDNA groups (SV and J; Table 2). These same female lineages are present on many other islands (Table 1). On Grenada, the southernmost island in the Lesser Antilles, we identified at least six female lineages representing all three of the mtDNA groups (SV, J, and G). Within the J mtDNA group, we found a bat in the J-1 lineage living on Grenada. Members of this same lineage also were found on every other island studied (Table 1). Likewise, within the SV group we found bats in the SV-1 lineage, which also constitute part of the *A. jamaicensis* populations on Barbados and St. Lucia and most of the population on St. Vincent (Table 1). Considering the high probability of lineage extinction, it is most likely that the discontinuities that separate the J, SV, and G groups resulted from evolution in geographic isolation (Avise et al. 1987). Thus, we think that the highly divergent mtDNA genotypes within the Grenada, St. Vincent, Barbados, and St. Lucia populations arrived independently from elsewhere and that these genotypes have been maintained by successful interisland movement of females, as documented by the presence of geographically wide-spread maternal lineages.

In contrast to the foregoing, studied populations in the northern Caribbean exhibited relatively little genetic diversity. Additionally, there is little direct evidence of frequent interisland movement although one could argue that broad distribution of bats in the J-1 lineage would require either a recent dispersal or maintainence through continuing interisland movement. Previous analysis of the J-1 mtDNA genotype with restriction enzymes that recognize 4-bp sequences failed to detect differences in the J-1 mtDNA isolated from bats living on Jamaica and St. Vincent, separated by nearly 1,400 km (unpubl. data).

All of the bats examined from the small island of Anguilla were in the same maternal lineage, J-1 (Table 1). The Anguilla population could have been founded exclusively by females in the J-1 lineage but alternatively other J lineages also might have reached the island, or originated there, but became extinct. Lineages from the SV and G mtDNA groups are also absent from Anguilla; in fact the nearest members of lineages in these divergent mtDNA groups live on St. Lucia and St. Vincent, more than 500 km to the south (Fig. 1; Table 1). At least two J lineages are represented on Jamaica. One, J-1, is found throughout the Caribbean but the other, J-2, is known only from Jamaica. The J-2 lineage might have originated on Jamaica, although its absence elsewhere could be due to chance extinction. Again, however, there is no evidence of lineages from either of the other mtDNA groups.

Intrapopulation (=intraisland) genetic heterogeneity ($\hat{h}$) can be estimated by formula [7] from Nei and Tajima (1981). On this basis Grenada is shown to exhibit the most heterogeneity (0.775) among the islands sampled; St. Lucia is second with 0.352 (Tables 1,3). Islands having several equally represented female lineages score much higher than islands on which the majority of the individuals carry one mtDNA genotype and the remaining few individuals represent several other lineages (e.g., St. Vincent versus St. Lucia, Table 3). Overall, the mean genetic heterogeneity for the eight islands sampled was 0.211. By way of comparison, Ashley and Wills (1987) reported that mtDNA heterogeneity in eight island populations of deer mice (*Peromyscus*) ranged from 0 to 0.44, with a mean of 0.20.

To some extent the foregoing data give a potentially misleading impression because Nei and Tajima's (1981) formula is based only on sample size and incidence (x_i) of each lineage in a population. Genetic heterogeneity on Jamaica (h = 0.189), where we found two J mtDNA genotypes with 0.4 percent sequence divergence, should be regarded in a totally different light than St. Vincent where bats trace their ancestries to three separate mtDNA groups with up to 13.9 percent sequence divergence but have a heterogeneity value of only h = 0.147 because one maternal lineage dominated the sample (Table 3).

The population of *Artibeus jamaicensis* on Grenada clearly is the most genetically diverse, both in terms of the incidence of different female lineages and the presence of divergent mtDNA groups. The genetic diversity in *A. jamaicensis* on Grenada can not be explained in terms of greater island size because Grenada is less than 50 percent the size of Jamaica or Puerto Rico. The most likely explanation is the geographic location of Grenada, which is only 150 km north of Trinidad and the South American mainland. Taken together, all of the mtDNA data suggest that *A. jamaicensis* on Grenada are not reproductively isolated and that new arrivals help to maintain considerable genetic diversity.

mtDNA and Antillean subspecies of *Artibeus jamaicensis*

In general, the colonization of the Antilles by *Artibeus jamaicensis* has followed a geographic pattern that is similar to the history hypothesized on the basis of analysis of exophenotypic features (Koopman 1968, present volume; Jones and Phillips 1970; Jones 1978, present volume; Baker and Genoways 1978). Four subspecies are thought to occur in the Caribbean. One, *A. j. parvipes*, occurs on Cuba and in the Bahamas but mtDNA has not yet been isolated from this subspecies. The second, *A. j. jamaicensis*, is widespread, occurring from Jamaica to St. Lucia and Barbados. *Artibeus j. schwartzi* occurs on St. Vincent and *A. j. trinitatus* occurs on Grenada south to Trinidad. In the northern

Antilles all 63 of our specimens of *A. j. jamaicensis* had a J mtDNA genotype but on St. Lucia, 79 percent of the *A. j. jamaicensis* carried another genotype, SV-1, as did 12 percent of the specimens collected on Barbados. The SV-1 mtDNA genotype is more typical (90 percent of the specimens) of *A. j. schwartzi* on St. Vincent (Table 1).

Overall, most specimens of *A. j. jamaicensis* (81%) have cytoplasmic genes in the J group. Animals carrying these genes are particularly interesting because of their broad geographic distribution and because of the small amount of sequence divergence discovered thus far in their mtDNA. Indeed, when the J-1 animals from Jamaica were compared to those living on Barbados (approximately 1,400 km apart), no differences were found with eight restriction enzymes (*Hin*d III, *Pvu* II, *Bam*H I, *Bgl* II, *Sal* I, *Eco*R I, *Pst* I, *Xba* I) that recognize different 6-bp sequences (Pumo et al. 1988). The relative uniformity in their mitochondrial genomes documents that these animals are closely related and suggests that this lineage has only recently spread throughout the Caribbean.

The possibility of recent dispersal and colonization by animals carrying the J mtDNA also is supported by paleontological evidence; Williams (1952) and Morgan and Woods (1986) have noted that on Jamaica and in the Cayman Islands, *Artibeus j. jamaicensis* is presently very abundant but is not found in Late Pleistocene or Early Holocene cave deposits. Instead, specimens are only known from the most superficial cave deposits. Likewise, specimens of *A. j. jamaicensis* are lacking from Pleistocene deposits in the Bahamas (Morgan, this volume). Subfossil specimens of *A. j. jamaicensis* from Puerto Rico (Reynolds et al. 1953; Choate and Birney 1968) have been collected from deposits in Cueva Monte Grande, Cueva de Clara, and Cueva del Perro. Although precise stratigraphic or radiocarbon dates are unavailable for these deposits, they are at least Pre-Columbian and possibly sub-Recent (Choate and Birney 1968) but unlikely to be Late Pleistocene or Early Holocene. Based on these data, we conclude that many of the *A. j. jamaicensis* living from Jamaica to Barbados are descendants of a common female ancestry and are quite likely the product of a recent and rapid dispersal over this large geographic area. We hypothesize that this dispersal was triggered by the gradual Holocene replacement of the xeric Pleistocene environment (dry grassland and thorn forest) by more mesic habitats with adequate fruit supply. Physiological and histological studies of *A. jamaicensis* collected in Panama lend support to this hypothesis. These investigations demonstrate that this species is not well-adapted to xeric environments; the kidney in *A. jamaicensis* is not subdivided as it is in xeric-adapted bats and urine concentrating ability is low (mean maximum of 972 mOsm/kg) (Studier et al. 1983a, 1983b). Typically, *A. jamaicensis* are dehydrated when they leave their roosts and depend on fruit juices for rapid rehydration. Finally, it is noteworthy that the recent dispersal of *A. jamaicensis* into the Caribbean also appears to correlate with Late Pleistocene extinctions and range reductions of several xeric-adapted chiropteran species living in the Greater Antilles (Morgan and Woods 1986).

The broad geographic distribution of *Artibeus j. jamaicensis* indicates that these bats have great dispersal power and are excellent colonizers. This is underscored by mtDNA data for the J group of genotypes. Part of their success must be derived from the fact that these bats are capable of flying distances up to 150 km (from St. Lucia to Barbados over open water). Other factors include reproduction, life span, mortality rate, and the carrying capacity of many of the islands. *Artibeus jamaicensis* probably have a bimodal reproductive cycle in the Antilles so females produce two young per year (Wilson 1979).

Per capita reproduction (λ) thus approaches two. For most island populations the value of K is very high, at least to judge from numbers of animals captured per night per mist net. While mortality rate (μ) is unknown, *A. jamaicensis* probably is not subject to predation on the islands. Part of the dispersal and colonization success of *A. jamaicensis*, documented by the mtDNA data for the J mtDNA genotypes, thus can be attributed to a high intrinsic rate of population increase (r), which is $= \lambda - \mu$ (MacArthur and Wilson 1967). It is noteworthy that probability of successful colonizing (r/λ), as predicted by MacArthur and Wilson's (1967) model, is high for *A. jamaicensis* and is borne out not only by its presence on many islands but also by the genetic history of *A. j. jamaicensis* on five islands spread over 1,400 km.

Artibeus j. schwartzi is a large-sized *A. jamaicensis* known from the island of St. Vincent, at the northern end of the Grenadines (Jones and Phillips 1970; Jones 1978). Our specimens from Bequia also can be assigned to this subspecies. Most of the specimens from St. Vincent (93 %) examined carried the SV-1 mtDNA genotype. One specimen had the J-1 mtDNA genotype and another carried the G-2 mtDNA genotype (Table 1). It is perhaps noteworthy that a fossil species, *Artibeus anthonyi*, named by Woloszyn and Silva Taboada (1977) from specimens collected in Cueva del Centenario de Lenin in Cuba, appears to be very similar, if not identical, to *A. j. schwartzi*. Woloszyn and Silva Taboada (1977) described the skull of *anthonyi* as similar in proportion to that of *A. lituratus* and presented cranial and mandibular measurements well within the range for *A. j. schwartzi* (Jones and Phillips 1970; Jones 1978). Is it possible that *A. j. schwartzi* formerly occupied a greater range in the Antilles?

Specimens of *Artibeus jamaicensis* from Grenada were originally named as a separate island subspecies, *A. j. grenadensis*, but later were assigned by Koopman (1968) to *A. j. trinitatus*. As discussed above, the mtDNA data from specimens collected on Grenada have revealed that these animals have an extremely complex genetic history.

Gene flow: are the Grenadines a filter?

Although gene flow generally has been regarded as a constraint to speciation (Mayr 1963), Slatkin (1987) has recently summarized conditions under which limited gene flow between populations might be a "creative" evolutionary force. Theoretical discussions of issues surrounding allopatric, sympatric, and peripatric speciation are common but the actual measurement of gene flow has rarely been achieved. Restriction endonuclease analysis of mtDNA offers one solution because by determining the geographic locations from which particular mtDNA genotypes have been recovered, it is possible to obtain direct documentation of gene flow between islands. Because it is considered unlikely that identical mtDNA lineages would evolve independently on multiple islands, the existence of the same lineage on more than one island is assumed to be due to movement of individuals from one place to another. Gene flow to an island is proportionate to colonization events so an island with four mitochondrial lineages probably has had considerably more divergent genetic input than has an island with but a single lineage. Only instances in which lineage extinction may have occurred would alter this conclusion. But the number of mtDNA lineages represents only a minimum number of founding events anyway.

The broad distribution of the J-1 mtDNA genotype documents recent gene flow among islands from Jamaica to Barbados. Likewise, the distribution of the J and SV mtDNA genotypes on St. Vincent, St. Lucia, and Barbados clearly shows gene flow among these islands. The differential incidence of particular mtDNA genotypes in different island populations might reveal something about gene flow but several other explanations also come to mind. For example, one could argue that incidence of a particular mtDNA genotype in a population could simply be the consequence of stochastic events. Alternatively, unequal distribution of a genotype might indicate recent arrival or might be the result of an asymmetric hybridization as has been discussed with regard to deer in the Southwest United States (Carr et al. 1986) and *Mus* in Scandinavia (Gyllensten and Wilson 1987).

Gene flow in the southern Lesser Antilles is particularly interesting because St. Vincent and Grenada are separated by both 120 km of water and the stepping stone-like Grenadines. A priori one might logically hypothesize that the Grenadines would facilitate gene flow between islands. However, the mtDNA data suggest just the opposite. The J mtDNA group, which has successfully spread throughout most of the Caribbean, is rare on St. Vincent and Grenada (Table 1). Animals carrying the J genotypes have dispersed successfully over a large area but from St. Vincent southward their genes are scarce. Animals carrying mtDNA genotypes in the G group have successfully colonized Grenada, which is about 130 km north of the South American mainland and Trinidad. However, only a single specimen (G-2, which also occurs on Grenada) has been found as far north as St. Vincent (Table 1). Bats carrying the SV mtDNA appear to be the only ones that have been very successful in moving between Grenada and St. Vincent.

An additional indication that the Grenadines have not facilitated gene flow comes from the distributional data for a related species, *Artibeus lituratus*. This large-sized fruit bat is relatively common throughout Middle America and northern South America. It also occurs on Trinidad and Grenada (Jones, this volume). Although it was thought to occur as far north as St. Vincent (Koopman 1968; Jones 1978), it is actually rare on that island. Jones and Phillips (1970) collected and reported on the only known specimen from St. Vincent. Phillips and Pumo (unpubl. data) did not capture any additional specimens during four nights of field work in 1986. This species is also absent from the northern Grenadine islands of Bequia and Mustique (Phillips and Pumo, unpubl. data). The Antillean population of *A. lituratus* thus is mostly confined to Grenada.

In summary, the genetic data as well as species distribution data suggest that the Grenadines have not facilitated movement of *Artibeus* though the Lesser Antilles, even though they provide an intermediate stepping stone connection. During the Pleistocene, Grenada and St. Vincent would have been nearly connected by a land bridge formed by the Grenadines. However, with the exception of bats carrying the SV-1 mtDNA, there is no indication that even this partial land bridge facilitated the movement of *Artibeus*. The Lesser Antilles, like the Greater Antilles, probably were xeric during the Pleistocene. Eshelman and Morgan (1985) have concluded that the late Pleistocene environment of near-by Tobago was similar to the present-day llanos (grassy plains) and dry thorn forest in South America. We postulate that northward colonization of the Lesser Antilles by *Artibeus* was influenced by the post-Pleistocene spread of more mesic habitats from northern South America into the Lesser Antilles (van der Hammen 1974). Current habitat conditions might also explain why the Grenadines have not served as a conduit for gene flow between the northern and southern Lesser Antilles. The Grenadines remain extremely xeric; many lack standing fresh water and fruit trees are nearly non-existent.

Taken collectively, these conditions do not appear suitable for ready colonization by *A. jamaicensis*. Unsuitable habitat probably is accentuated by the small size of the individual Grenadines. As Whitehead and Jones (1969) have pointed out in their discussion of small islands and equilibrium theory, island size is very likely to exert influence over colonization rate. A combination of small size and marginal habitat would result in an extremely small K value (perhaps essentially zero) for a resident population and, based on MacArthur and Wilson's (1967) mathematical analysis of survivorship, permanent populations would easily go extinct without new arrivals. New arrivals would recolonize rather than compete or hybridize with an existing population. The picture that emerges for the Grenadines is one in which these small, dry islands might have been colonized repeatedly but rarely served as a source population for continuing dispersal. The mtDNA data show that on average fruit bats of the genus *Artibeus* have been more successful at crossing expanses of open ocean than moving through the Grenadines. It is possible that bats in the SV-1 mtDNA lineage were slightly better suited to survival in xeric conditions and were able to colonize Grenada and St. Vincent during the Pleistocene. Perhaps these bats are derived from a stock that reached the Antilles earlier than others and this could explain the evolution of the morphologically distinctive *A. j. schwartzi*.

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Table 1. Summary of specimens examined, localities, and mtDNA genotypes. The genotypes were assigned to one of three groups, designated J, SV, and G, rather than to specific maternal lineages. See text for details.

	Principal mtDNA Genotypes (% and sample size)			
	J	SV	G	TOTALS
A. j. jamaicensis				
Jamaica	100 (21)	0	0	21
Puerto Rico	100 (33)	0	0	33
Anguilla	100 (9)	0	0	9
Barbados	88 (15)	12 (2)	0	17
St. Lucia	21 (6)	79 (23)	0	29
				109
A. j. schwartzi				
St. Vincent	5 (1)	90 (18)	5 (1)	20
Bequia	0	100 (7)	0	7
				27
A. j. trinitatus				
Grenada	3.6 (1)	36 (10)	60 (17)	28
	Total specimens examined			164

Table 2. Estimates of percent nucleotide sequence divergence in pairwise comparisons of mtDNA lineages isolated from Antillean *Artibeus jamaicensis*. Upper diagonal: percent sequence divergence calculated with Nei and Li's (1979) equation 16 and alpha = 2. Lower diagonal: percent nucleotide sequence divergence, standard deviation, and variance (in parentheses) calculated with formulae 9 and 11, Nei and Tajima (1983).

	J-1	J-2	SV-1	G-1	G-2
J-1	---	0.4	9.3	16.7	17.2
J-2	0.4 ± 0.4 (0.001)	---	11.0	15.0	15.5
SV-1	8.2 ± 2.2 (0.05)	9.5 ± 2.6 (0.06)	---	16.1	16.7
G-1	13.5 ± 3.7 (0.12)	12.4 ± 3.3 (0.11)	13.1 ± 3.4 (0.12)	---	0.4
G-2	13.9 ± 3.5 (0.12)	12.8 ± 3.4 (0.11)	13.5 ± 3.5 (0.12)	0.4 ± 0.4 (0.002)	---

Table 3. Intrapopulation genetic variations, $\hat{h}$ (based on mtDNA lineage data; Nei and Tajima, 1981*) in *Artibeus jamaicensis* on eight islands. Islands are listed in order of decreasing area.

Island	Sample size	Minimum No. mtDNA group(s) J	SV	G	Genetic mtDNA lineages	variation ($\hat{h}$)
Jamaica	21	x			2	0.189
Puerto Rico	33	x			1	0
St. Lucia	29	x	x		2	0.352
Grenada	27	x	x	x	6	0.775
St. Vincent	20	x	x	x	3	0.147
Barbados	19	x	x		2	0.221
Anguilla	9	x			1	0
Bequia	7		x		1	0

*
$$\hat{h} = \frac{N}{N-1}\left(1 - \sum_i x_i^2\right)$$

N is sample size; x_i is frequency of the *i*th lineage

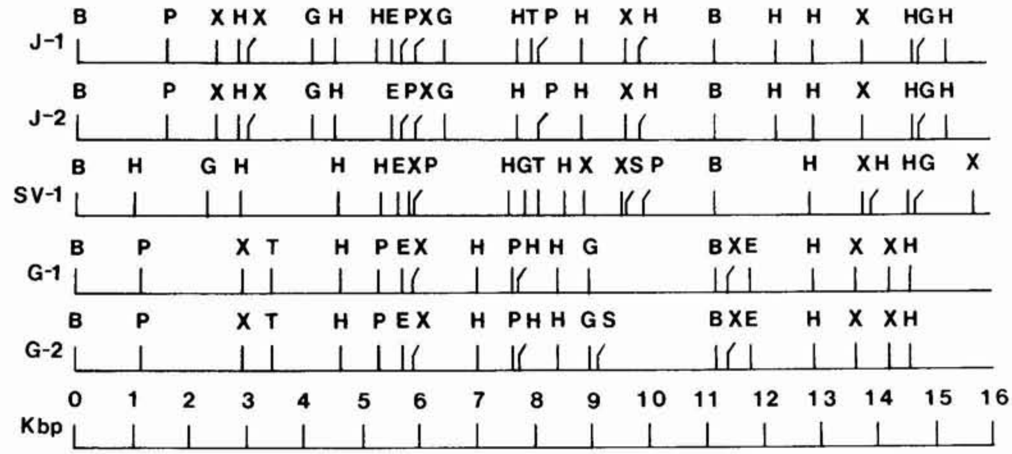

Figure 1.--Restriction maps of five mitochondrial DNA genotypes of *Artibeus jamaicensis* collected in the West Indies. All maps have been linearized at a conserved *Bam*H I site. Enzyme recognition sites are coded as follows: B, *Bam*H I; H, *Hind* III; E, *Eco*R I; P, *Pvu* II; G, *Bgl* II; T, *Pst* I; S, *Sal* I; X, *Xba* I. J-2 has not been tested with *Pst* I.

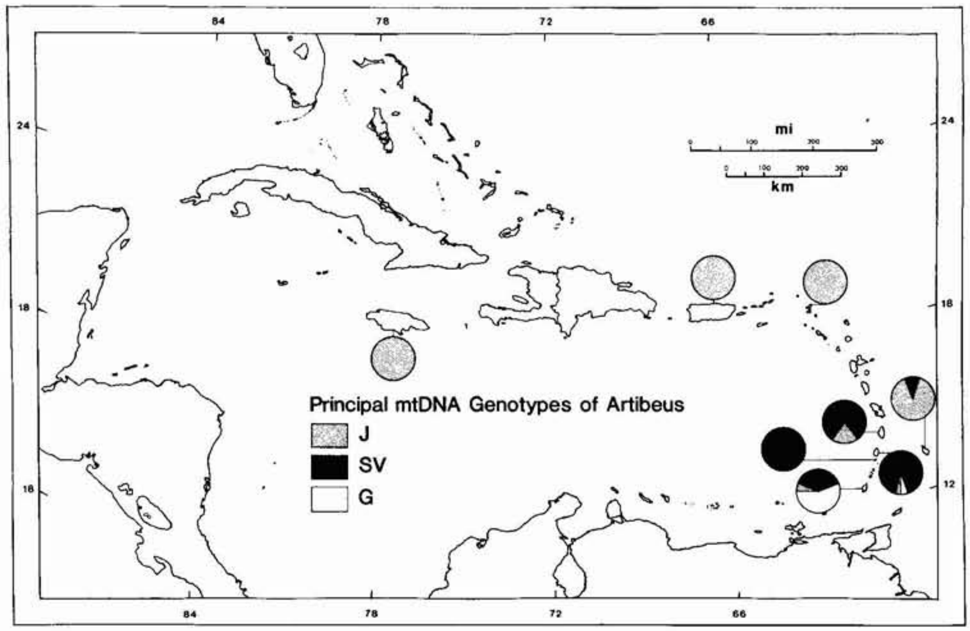

Figure 2.--Map of the Caribbean showing the relative incidence of genotype groups (J, SV, and G) on the islands in this report. Note that only the J group is found throughout the Caribbean.